Attorney Docket No.: 01-053120US
Client Reference No.: 100/10210US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

U.S. PATENT APPLICATION FOR

METHODS AND DEVICES FOR DISPERSION-CONTROLLED TRANSPORT OR PROCESSING OF POLAR OR POLARIZABLE MATERIALS

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As Filed: **FRIDAY, NOVEMBER 16, 2001**

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METHODS AND DEVICES FOR DISPERSION-CONTROLLED TRANSPORT AND PROCESSING OF POLAR OR POLARIZABLE MATERIALS

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CROSS-REFERENCES TO RELATED APPLICATIONS

[0002] Pursuant to 35 U.S.C. §§ 119 and/or 120, and any other applicable statute or rule, this application claims the benefit of and priority to USSN 60/249,737, filed on November 17, 2000, the disclosure of which is incorporated by reference.

BACKGROUND OF THE INVENTION

[0003] Throughput is the rate at which work is performed in a given system. Improvements to the capabilities of analytical instrumentation, such a microfluidic devices, are often made by enhancing device throughput. One way to elevate the throughput of various microfluidic applications is to increase the number of discrete samples or "slugs" of material accommodated per unit length of a given microchannel or other microscale device cavity. Limiting factors such as slug dispersion and diffusion, which are typically irreversible processes, restrict minimum slug-to-slug spacing in addition to time averaged sample concentrations. As a result, microfluidic devices in which slug dispersion and diffusion are either inhibited or reversible in a controllable manner would further improve the throughput and performance of various microfluidic analytical and preparative processes.

[0004] It is generally known that localized electric fields may be used to capture polar or polarizable materials. Asbury and van den Engh (1998) "Trapping of DNA in Nonuniform Oscillating Electric Fields," Biophys. J. 74:1024-1030 demonstrated

that materials, such as polynucleotides may be subjected to dielectrophoretic forces strong enough to concentrate them within localized electric fields (e.g., along the edges of thin metal electrodes). In particular, due to the induction of electric dipoles, nucleic acids were pulled by a gradient force to regions of high electric field strength. The molecules were captured locally in an oscillating field using thin strips of gold film to generate strong electric fields with steep gradients. Additionally, spatial control over the captured polynucleotides was achieved with the molecules being confined to a narrow width along the edges of the gold strips.

[0005] Dielectrophoresis generally includes the lateral motion imparted on uncharged particles as a result of polarization induced by non-uniform electric fields. *See,* Pohl, Dielectrophoresis, Cambridge University Press, Cambridge (1978). For example, when a spatially non-uniform electric field is applied to a system that includes dielectric particles suspended in a liquid medium, the applied field typically induces a dipole moment in each particle as a result of electrical polarizations forming at the interfaces that define their structures. The interaction of the induced dipole with the electric field generates a force. In the presence of the field gradient, these forces are generally not equal and there is a net movement. If the particle is more conductive than the medium around it, the dipole aligns with the field and the force acts up the field gradient towards the region of highest electric field (i.e., positive dielectrophoresis). On the other hand, if the particle is less polarizable than the surrounding medium, the dipole aligns against the field and the particle is repelled from regions of high electric field (i.e., negative dielectrophoresis). *See,* Pethig (1996) "Dielectrophoresis: Using Inhomogeneous AC Electrical Fields to Separate and Manipulate Cells" Critical Reviews in Biotechnology 16:331-348. The force is dependent on the induced dipole, not on the direction of the electric field, and responds only to the field gradient. Additionally, since the field alignment is immaterial, this force is typically generated in alternating current fields which has the advantage of reducing electrophoretic forces to zero. By carefully designing electrode geometries, which create the localized electric fields, it is possible to produce electric field differences such that potential energy minima are bounded by regions of increasing electric field strengths. In such fields, particles experiencing positive dielectrophoresis are attracted to the regions of highest electric field (typically at

the electrode edges), while particles experiencing negative dielectrophoresis are trapped in isolated field minima.

[0006] Many different dielectrophoretic applications (e.g., material manipulation and transport) are generally known and described in the literature, including, e.g., Pohl (1958) "Some Effects of Nonuniform Fields on Dielectrics," J. Appl. Phys. 29:1182-1188, Wang et al., (1993) "Selective Dielectrophoretic Confinement of Bioparticles in Potential Energy Wells," J.Phys.D: Appl.Phys. 26:1278-1285, Talary et al., (1995) "Dielectrophoretic Separation and Enrichment of CD34 Cell Subpopulation from Bone Marrow and Peripheral Blood Stem Cells," Med. & Biol. Eng. & Comp. 33:235-237, Bakewell et al., (1998) "Dielectrophoretic Manipulation of Avidin And DNA" Proc. 20th Annual International Conference of the IEEE Engineering in Medicine and Biology Society, Batchelder (1983) "Dielectrophoretic manipulator" Rev. Sci. Instrum 54:300-302, Fuhr et al., (1994) "Travelling-wave driven microfabricated electrohydrodynamic pumps for liquids" J. Micromech. Microeng. 4:217-226, Fuhr G et al., (1994) "Particle micromanipulator consisting of two orthogonal channels with travelling-wave electrode structures" Sensors and Actuators A 41-42:230-239, Green and Morgan (1997) "Dielectrophoretic separation of nano-particles" J. Phys. D: Appl. Phys. 30:L41-L44, Green et al., (1997) "Manipulation and trapping of sub-micron bioparticles using dielectrophoresis" J. Biochem Biophys. Methods 35:89-102, Green et al., (1999) "Sub-micrometre AC electrokinetics: particle dynamics under the influence of dielectrophoresis and electrohydrodynamics" Inst. Phys. Conf. Ser. 163:89-92, Hagedorn et al., (1994) "Design of asynchronous dielectric micromotors" J. Electrostatics 33:159-185, Huang and Pethig (1991) "Electrode design for negative dielectrophoresis" Meas. Sci. Technol. 2:1142-1146, Hughes and Morgan (1998) "Dielectrophoretic Manipulation of Single Sub-micron Scale Bioparticles" J. Phys. D: Appl. Phys. 31:2205-2210, Schnelle et al., (1999) "Paired microelectrode system: dielectrophoretic particle sorting and force calibration" J. Electrostatics 47:121-132, Huang et al., (1993) "Electrokinetic behavior of colloidal particles in travelling electric fields: studies using yeast cells," J. Phys. D: Appl. Phys. 26:312-322, Müller et al., (1996) "Trapping of micrometre and sub-micrometre particles by high-frequency electric fields and hydrodynamic forces" J. Phys. D: Appl.

Phys. 29:340-349, and Washizu et al., (1994) "Molecular dielectrophoresis of biopolymers" IEEE Trans. Ind. Appl. 30:835-842.

[0007] In general, additional methods for increasing microfluidic device throughput would be desirable. The present invention provides methods and devices for reversibly controlling dispersion of materials during the flow (e.g., pressure-based flow) of fluidic materials in microfluidic devices to thereby enhance device throughput. The methods include capturing polar or polarizable particles in localized electric fields generated by alternating (AC) or direct currents (DC). These and a variety of additional features and applications will become apparent upon complete review of the following description.

SUMMARY OF THE INVENTION

[0008] The present invention generally relates to methods for controlling dispersion of materials during fluid transport within microscale cavities of microfluidic devices. In particular, the invention provides methods, devices, and integrated systems for reversibly capturing material in localized electric fields, including dielectrophoretically-induced fields. During capture, many different processes are optionally performed, including nucleic acid amplification and sequencing. These procedures are optionally followed by various downstream processing steps such as reagent separation and/or detection. Among the advantages provided by the invention is the capacity to reversibly regulate the dispersion of materials within discrete fluid samples. Previously, such dispersion was essentially an irreversible process that severely limited the minimum spacing possible between adjacent discrete fluid samples flowed in the devices and thus, device throughput.

[0009] The methods of controlling dispersion of material in a microfluidic device, include flowing the material (e.g., a discrete sample thereof), e.g., under pressure in a microchannel of the microfluidic device and capturing the material in at least one localized electric field generated in a portion of the microchannel. For example, the methods include flowing a fluid continuously through the microchannel during operation of the microfluidic device using a fluid pressure force modulator or the like. The methods also include releasing the material from the localized electric field in the portion of the microchannel. The releasing step typically includes deactivating the localized

electric field during pressure-based flow of at least one fluid (e.g., a solvent, an electrolyte, a buffer, or the like) through the portion of the microchannel. The material typically includes a polar or polarizable material. In particular, the material generally includes a polarity or polarizability that is greater than a polarity or polarizability of a fluid (e.g., a solvent, a buffer, an electrolyte, or the like) that includes the material. In preferred embodiments, the methods further include repeating one or more of the flowing, capturing, or releasing steps at least once in which each time the capturing step is repeated, the material is captured in at least one other localized electric field in at least one other portion of the microchannel that is downstream from preceding capturing step portions. The methods optionally include controlling the dispersion of a plurality of discrete samples of the material by sequentially introducing each discrete sample into the microchannel following at least one cycle of the flowing, capturing, and releasing steps.

[0010] Materials are captured according to the methods of the present invention using various approaches. For example, the localized electric field is optionally generated by at least one alternating current source or by at least one direct current source. Further, the localized electric field is capable of being selectively activated or deactivated. In certain embodiments, the material is dielectrophoretically captured in the localized electric field. The capturing step generally includes activating the localized electric field during (i.e., simultaneous with), e.g., pressure-based flow of the material through the portion of the microchannel to effect capture, and is typically a reversible step. Optionally, lines of force of the localized electric field are directed substantially vertically or substantially horizontally in the portion of the microchannel. In certain embodiments, the methods also include altering one or more dielectrophoretic properties of the material prior to performing the capturing step. For example, dielectrophoretic properties of the material are optionally altered by attaching each material component to separate microbeads.

[0011] The portion of the microchannel in which materials are captured typically includes a capture electrode configuration for generating the localized electric field. In certain embodiments, the capture electrode configuration includes at least one capture electrode disposed between a first field electrode portion and a second field electrode portion in which at least a segment of the capture electrode, and the first and

second field electrode portions are partially disposed within the microchannel. Additionally, an electrical control system is typically configured to permit current (e.g., AC, DC, or both) to flow through at least one fluid disposed between the first and second field electrode portions in the microchannel. Optionally, capture electrodes are directly or indirectly connected to an electrical control system and typically include higher conductivities than fluid which includes the materials in the portion of the microchannel. For example, capture electrodes or the first and second field electrode portions are generally fabricated from at least one metallic substance (e.g., Au, Pt, alloys, or the like).

[0012] In preferred embodiments, the capture electrode configuration includes multiple capture electrodes in which each capture electrode is disposed at least partially in or proximal to a different portion of the microchannel and is capable of generating at least one separate localized electric field. Optionally, each of the multiple capture electrodes is regularly or irregularly spaced from one another along a length of the microchannel. For example, each of the multiple capture electrodes is optionally disposed at least about one μm from one another along a length of the microchannel.

[0013] In other embodiments, the capture electrode configuration includes at least one capture electrode pair. For example, capture electrode pairs optionally include a first capture electrode disposed partially in or proximal to a top portion of the microchannel and a second capture electrode disposed partially in or proximal to a bottom portion of the microchannel in which the top and bottom portions oppose each other in the microchannel. Alternatively, capture electrode pairs include a first capture electrode disposed partially in or proximal to a first side portion of the microchannel and a second capture electrode disposed partially in or proximal to a second side portion of the microchannel in which the first and second side portions oppose each other in the microchannel. As a further alternative, capture electrode pairs include a first capture electrode and second capture electrode, both disposed in or proximal to one top, bottom, or side portion of the at least one microchannel. When multiple capture electrode pairs are provided in the same device, any combination of these pairs are optionally included. Typically, at least one segment of each electrode in a capture electrode pair substantially oppose each other in the at least one microchannel. Additionally, each electrode of a capture electrode pair is generally connected to an electrical control system, e.g., for

selectively flowing AC and/or DC to electrode pairs for generating localized electric fields.

[0014] In certain preferred embodiments, the capture electrode configuration includes multiple capture electrode pairs in which each electrode of each capture electrode pair is disposed at least partially in or proximal to a different portion of the microchannel. Each of the multiple capture electrode pairs is capable of generating a separate localized electric field. Additionally, each of the multiple capture electrode pairs is regularly or irregularly spaced from one another along a length of the microchannel. For example, each of the multiple capture electrode pairs is optionally disposed at least about one μm from one another along a length of the microchannel.

[0015] The methods typically include flowing the material into the microchannel from a cavity (e.g., an additional microchannel, a port, a capillary element that extends from the microfluidic device, or the like) that fluidly communicates with the microchannel. The methods also optionally include pinching fluid flow in the microchannel from at least one direction upon introduction of the material into the microchannel. For example, the pinching and capturing steps optionally occur substantially at the same time.

[0016] During capture of materials in localized electric fields many different processes are optionally performed. For example, when each of the multiple captured discrete samples include a plurality of captured first nucleic acids, the methods optionally include flowing a set of second nucleic acids, e.g., under pressure into the microchannel, hybridizing the set of second nucleic acids to the plurality of captured first nucleic acids to provide a set of hybridized nucleic acids, and elongating the set of hybridized nucleic acids (e.g., with at least one thermostable polymerase, reverse transcriptase, or ligase). These steps, which typically occur in a nucleic acid amplification process (e.g., a polymerase chain reaction, a ligase chain reaction, or the like) or in a nucleic acid sequencing process, also optionally include denaturing the set of hybridized nucleic acids to provide a set of denatured nucleic acids, rehybridizing the set of denatured nucleic acids to provide a set of further hybridized nucleic acids, and extending the set of further hybridized nucleic acids (e.g., with at least one thermostable polymerase, reverse transcriptase, or ligase). A further option includes repeating the

denaturing, rehybridizing, and extending steps at least once. The methods generally include controlling temperature within the microchannel by using at least one selectable heating technique (e.g., joule heating, zone heating, a hot plate, or the like). For example, temperature is optionally controlled within the microchannel with at least one joule heating electrode disposed in at least one microscale cavity that fluidly communicates with the microchannel, which joule heating electrode is operably connected to a current source. In certain embodiments, at least a portion of the microscale cavity is wider than a cross-section of at least a portion of the microchannel. Disposing joule heating electrodes in microscale cavities that fluidly communicate with the microchannel (i.e., are not directly disposed within the microchannel) further controls the dispersion of material in the microfluidic device.

[0017] When materials (e.g., multiple discrete samples) are captured in localized electric fields many other assays or processes are also optionally performed. For example, the methods optionally include flowing at least one additional material through the microchannel that includes the multiple captured discrete samples of the material. The methods generally include detecting a detectable signal produced by one or more interactions of the additional material with one or more of the multiple captured discrete samples of the material. For example, the methods optionally include comparing the detectable signal with at least one standard. In certain embodiments, the methods also include separating one or more of the material, the additional material, or products of the one or more interactions.

[0018] The present invention also provides methods of amplifying nucleic acids. The methods include positioning a set of first nucleic acids in a portion of a microchannel, flowing a set of second nucleic acids, e.g., under pressure into the microchannel, and capturing the set of first nucleic acids in a localized electric field generated in the portion of the microchannel. The methods also include hybridizing the set of second nucleic acids to the set of captured first nucleic acids to provide a set of hybridized nucleic acids and elongating the set of hybridized nucleic acids (e.g., with a thermostable polymerase, reverse transcriptase, or ligase) to amplify the nucleic acids. These steps typically occur in one or more polymerase chain reactions or one or more ligase chain reactions.

[0019] The present invention also provides a microfluidic device that includes a substrate having a surface and at least one microchannel fabricated into the surface of the substrate in which the microchannel includes at least one capture electrode configuration. The device also includes a source of a material in fluid communication with the microchannel, an electrical control system (e.g., a selectable electrical field source, an alternating current source, a direct current source, and/or an arbitrary current source) operably connected to the at least one capture electrode configuration for generating at least one localized electric field in the at least one capture electrode configuration to reversibly capture the material, a pressure-based fluid direction system (e.g., a fluid pressure force modulator or the like) operably connected to the microfluidic device for inducing flow of the material in the microchannel, and a cover mated with the surface of the substrate. In certain embodiments, the at least one microchannel of the microfluidic device includes a plurality of parallel microchannels fabricated into the surface of the substrate in which each parallel microchannel includes at least one capture electrode configuration. In other embodiments, the microfluidic device further includes at least one joule heating electrode disposed in at least one microscale cavity that fluidly communicates with the at least one microchannel, which joule heating electrode is operably connected to the at least one electrical control system to control temperature within the at least one microchannel. Optionally, at least a portion of the at least one microscale cavity is wider than a cross-section of at least a portion of the at least one microchannel.

[0020] The microfluidic devices of the present invention also typically include an integrated system. The system generally includes a computer or a computer readable medium that includes at least one instruction set for selectively activating or deactivating the at least one localized electric field in the at least one material capture portion and a controller/detector apparatus configured to receive the microfluidic device. The controller/detector apparatus typically includes a detection system and a material transport system in which the detection and transport systems are operably interfaced with the microfluidic device.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] Figure 1A schematically shows a microchannel configuration for dispersion-controlled pressure-based material transport.

[0022] Figure 1B schematically illustrates the dispersion-controlled transport of a captured discrete sample of material.

[0023] Figure 1C schematically shows relative electric field strengths between captured and released states.

[0024] Figure 1D schematically illustrates the attachment of a material component to a microbead to alter the dielectrophoretic properties of the material.

[0025] Figure 2A schematically shows a microchannel configuration for pressure pumped dielectrophoretic material transport that includes an electrical control system having an XOR connection between AC and DC sources.

[0026] Figure 2B schematically illustrates one embodiment of a method of injecting discrete samples of material into microchannels for subsequent dispersion-controlled material transport and/or for other processing or analysis.

[0027] Figures 3A-3C schematically depicts embodiments of capture electrode configurations that include capture electrode pairs.

[0028] Figure 4 schematically shows a capture electrode pair with vertically directed lines of force.

[0029] Figure 5A schematically illustrates a linear array of capture electrode pairs.

[0030] Figure 5B schematically illustrates a two-dimensional array of capture electrode pairs.

[0031] Figure 6A schematically depicts a microchannel configuration that includes parallelized capture electrode configurations, e.g., for nucleic acid amplification and/or sequencing.

[0032] Figure 6B schematically shows a magnified view of one capture electrode from the capture electrode configuration of Figure 6A that includes positioned nucleic acids embedded in meltable agarose gel.

[0033] Figure 6C schematically illustrates a method for positioning materials, such as nucleic acids on selected capture electrodes.

[0034] Figures 7A-7C schematically show a microfluidic device that includes a capillary element from various viewpoints.

[0035] Figure 8 schematically illustrates a integrated system that includes the microfluidic device of Figures 7A-7C.

DETAILED DISCUSSION OF THE INVENTION

INTRODUCTION

[0036] The present invention generally provides improved methods, and related devices, for reversibly controlling dispersion of materials in microfluidic devices. In particular, selected amounts of polar or polarizable materials are sequentially captured and released from, e.g., a series of localized electric fields during flow (e.g., pressure-based flow) in microchannels or other microfluidic device cavities to prevent dispersion of materials between discrete sample volumes. In addition, while materials are captured in the localized electric fields, assorted processes are optionally performed, including the amplification or sequencing of nucleic acids, the assessment of binding or other molecular interactions, or the like. The invention also provides various alternative formats for generating localized electric fields within the devices. Additionally, integrated systems are also provided.

[0037] As used herein, the phrase "localized electric field" refers to a region of a microchannel or other microfluidic device cavity that includes a higher electric field than adjacent regions. For example, a localized electric field is optionally generated at a specific location of the microchannel or other cavity for capturing polar or polarizable materials, such as polynucleotides, polypeptides, or the like. In certain embodiments, localized electric fields are produced by disposing capture electrodes (e.g., metallic foil, metal wires, deposited conductive coatings, or other material that has higher conductivity than a surrounding fluidic medium) between first and second field electrode portions located in a microchannel. Upon flowing alternating current between the two field electrode portions, the capture electrodes act as short circuits such that the applied electric field is concentrated by the capture electrodes to create field gradients. In positive dielectrophoresis, the applied current induces dipoles in polar or polarizable material such that gradient forces pull the material to the localized electric fields or

regions of higher electric field strength generated at the capture electrodes. The flow of alternating current between the first and second field electrode portions is typically selectively activated or deactivated. The capture electrodes are optionally directly or indirectly connected to external power sources. *See also,* Asbury and van den Engh (1998) "Trapping of DNA in Nonuniform Oscillating Electric Fields" Biophys. J. 74:1024-1030 for an illustration of indirectly connected capture electrodes. In other embodiments, localized electric fields are generated in capture electrode configurations that include, e.g., capture electrode pairs. In these embodiments, each electrode of a capture electrode pair is typically electrically connected to an external power source, such as a DC and/or AC source which is optionally selectively activated or deactivated. Localized electric fields are generated between the electrodes of a given capture electrode pair to effect capture of polar or polarizable materials.

[0038] One particular advantage of the methods of reversibly controlling the dispersion of materials during flow (e.g., pressure-based flow) is improved device throughput. For example, when transporting discrete samples of materials in microfluidic devices, spacer fluid is typically flowed between adjacent samples as a barrier to the dispersion of materials between samples. The methods of the invention increase device throughput by effectively decreasing the volumes of spacer fluid used to prevent dispersion such that for given sample volumes, more samples are transported per unit microchannel length without dispersion among individual sample volumes than is possible in other devices.

[0039] The following provides details regarding various aspects of the methods of controlling dispersion of materials during fluid flow in microfluidic devices, including alternative capturing formats, capture electrode configurations, localized electric field generation, and fluid flow in microfluidic devices. It also provides details pertaining to the methods of amplifying nucleic acids and to the high-throughput integrated systems that are typically used to control the dispersion of materials and to perform other upstream and/or downstream steps, including assay detection.

DISPERSION-CONTROLLED MICROFLUIDIC MATERIAL TRANSPORT METHODS

[0040] The present invention provides many different approaches to effect the dispersion-controlled transport of materials in microfluidic devices. For example, materials are optionally captured or focused in localized electric fields generated by AC and/or DC power sources. Localized electric fields are optionally generated using various capture electrode configurations that are either directly or indirectly connected to electrical control systems. The invention also includes methods of introducing or injecting discrete sample volumes into microchannels, e.g., for subsequent dispersion-controlled transport and/or analysis. Furthermore, the methods include altering certain properties, such as dielectrophoretic properties, of materials (e.g., polynucleotides, polypeptides, or the like) to provide additional levels of control over dispersion processes.

[0041] As used herein, the term "dispersion" refers to diffusion and to the convection-induced, longitudinal dispersion of material within a fluid medium due to velocity variations across streamlines, e.g., in pressure driven flow systems, electrokinetically driven flow systems around curves and corners, and electrokinetically driven flow systems having non-uniform buffer ionic concentrations, e.g., plugs of high and low salt solutions within the same channel system. For the purposes of the channel systems of the present invention, dispersion is generally defined as that due to the coupling between flow and molecular diffusion, i.e., Taylor dispersion. In this regime, the time-scale for dispersion due to convective transport is long or comparable to the time scale for molecular diffusion in the direction orthogonal to the flow direction. For discussions on dispersion and Taylor dispersion in particular, see, e.g., Taylor et al., Proc. Roy. Soc. London (1953) 219A:186-203, Aris, Proc. Roy. Soc. London (1956) A235:67-77, Chatwin et al., J. Fluid Mech. (1982) 120:347-358, Doshi et al., Chem. Eng. Sci. (1978) 33:795-804, and Gnell et al., Chem. Eng. Comm. (1987) 58:231-244, each of which is incorporated herein by reference.

[0042] The methods of controlling the dispersion of materials (e.g., polar materials, polarizable materials, or the like) in a microfluidic device generally include flowing the materials (e.g., multiple discrete samples of material) under pressure, electrokinetically, via other flow methods, or combinations thereof in a microchannel of

the device and reversibly capturing the materials in localized electric fields generated in a portion of the microchannel. Pressure-based flow embodiments are preferred. In particular, the material typically includes a polarity or polarizability that is greater than a polarity or polarizability of a surrounding fluidic medium (e.g., a solvent, a buffer, an electrolyte, or the like) that includes the material. The methods typically include flowing the material into the microchannel from a cavity (e.g., an additional microchannel, a port, a capillary element that extends from the microfluidic device, or the like) that fluidly communicates with the microchannel. Additionally, the methods typically include flowing a fluid, e.g., continuously through the microchannel during operation of the microfluidic device, e.g., using a fluid pressure force modulator or the like. The flow of materials in the devices of the invention is described in greater detail below.

[0043] Materials are captured according to the methods of the present invention utilizing various approaches. In particular, localized electric fields are optionally generated by at least one alternating current source and/or by at least one direct current source. For example, in certain embodiments, materials are dielectrophoretically captured in the localized electric fields. In addition, the localized electric fields are generally capable of being selectively activated or deactivated. To illustrate, the capturing step typically includes activating one or more localized electric fields during (i.e., simultaneous with) flow of the materials through a microchannel or other cavity to effect capture. The generation of localized electric fields and alternative capture electrode configurations are described further below.

[0044] Following capture, the dispersion-controlled transport methods typically include releasing the captured materials from the localized electric fields. The methods generally include repeating the cycle of flowing, capturing, and releasing steps multiple times in which each time the capturing step is repeated, the material is captured in another localized electric field in another portion of the microchannel that is downstream from preceding capturing step portions. The releasing step typically includes deactivating one or more of the localized electric fields during, e.g., pressure-based flow of a fluid through the microchannel to permit fluid drag forces to move the material further downstream. In certain embodiments, the methods optionally include controlling the dispersion of a plurality of discrete samples of the material by sequentially

introducing each discrete sample into the microchannel following at least one cycle of the flowing, capturing, and releasing steps. For example, at least two of the plurality of discrete samples of the material typically include different constituent materials or different sample sizes. Furthermore, the methods typically include introducing a selected volume of a spacer fluid (e.g., a buffer, a solvent, or the like) into the microchannel following the introduction of each sample of the material into the microchannel to provide a fluid barrier between each adjacent pair of the discrete samples.

[0045] Figure 1A schematically shows one embodiment of a microchannel configuration used in the pressure-pumped dielectrophoretic material transport methods described herein. As shown, microfluidic device **100** includes a cross microchannel configuration in which one of the microchannels includes series of equidistantly spaced metal capture electrodes **102**. In other embodiments, the capture electrodes are irregularly spaced. Although not shown, a four microchannel vacuum pumping system for applying pressure-based flow is also typically operably connected to the device via wells **104**, **106**, **108**, and **110**. In addition, two of the wells (namely, **106** and **110**) include field electrode portions (**112** and **114**, respectively) for the application of alternating current from electrical control system **116**.

[0046] During the application of alternating current through the microchannel that includes series of equidistantly spaced metal capture electrodes **102**, the dielectrophoretic force on polar or polarizable materials (e.g., polynucleotides or the like) in the fluidic medium will typically exceed the drag force the materials experience due to pressure-based fluid flow in the microchannel channel to effect material capture in the localized electric fields at each metal capture electrode. (FIG. 1A). For example, dielectrophoretic forces have been estimated to be in the femtonewton range for relatively high molecular weight nucleic acids, such as lambda DNA, e.g., by applying the following equation:

$$F = 2\pi r^3 \varepsilon_m \, \mathrm{Re} \, \{f(\varepsilon^*_p, \varepsilon^*_m) \, \nabla E^2\}$$

where r is the radius of the particle, ε_m is the permittivity of the suspending medium, Re is the Reynolds number, ∇ is the Del vector, E is the electric field strength, and $f(\varepsilon^*_p, \varepsilon^*_m)$ is the Clausius-Mossotti factor, which defines the dielectric properties of the particle and the suspending medium in terms of their respective complex permittivities ε^*_p and ε^*_m.

See, e.g., Asbury and van den Engh (1998) "Trapping of DNA in Nonuniform Oscillating Electric Fields" Biophys. J. 74:1024-1030. In contrast, fluid drag forces are estimated, e.g., by applying the equation $F = 6\pi r\eta v$, where r is the radius of the particle or material component, η is the viscosity of the medium, and v is flow speed.

[0047] Figure 1B schematically illustrates the transport or translocation of a captured discrete sample of material (i.e., a "slug" of material) according to the methods described herein. The device depicted in Figure 1B is a magnified view of the intersection of microchannels shown in Figure 1A with material captured on first capture electrode **118** and no material captured on second capture electrode **120**. Electrical control system **116** of Figure 1B includes switch **122** for delivering pulses of AC in the microchannel between field electrode portions **112** and **114**. In other embodiments, electrical control systems include XOR connections between AC and DC sources to produce cycles of AC and DC pulses to effect dispersion-controlled transport. *See, e.g.,* Figure 2A. Figure 1B also includes schematic current plot **124** that depicts three cycles **126** of AC activation **128** and deactivation **130**. During AC activation **128**, material is captured, e.g., as shown at first capture electrode **118**, whereas during AC current deactivation **130**, material is released from the electrodes and flows downstream due to fluid drag forces during which time the material will diffuse and disperse until current is activated in a subsequent cycle.

[0048] Cycles of AC activation and deactivation are further depicted in Figure 1C, which includes a magnified view of first capture electrode **118** with captured material and second capture electrode **120** from Figure 1B. Figure 1C also includes electric field strength plot **132** with increasing field strength indicated by arrow **134**. Arrow **136** indicates the direction of fluid flow in the microchannel. As shown, material is captured during AC activation (represented by, e.g., corresponding potential well **138**) and released from first capture electrode **118** when the AC is deactivated. Arrow **140** represents the time following AC deactivation during which the material slug is carried out of the steep potential well and escapes from the edges of first capture electrode **118**. That is, fluid drag forces produced by the constant fluid pressure pumping will rapidly overcome any residual dielectrophoretic forces at short distances away from the electrode. AC pulse spacing is generally correlated with the fluid flow rate in the

microchannel, but the precise timing relationships are typically not essential. Arrow **142** indicates the time during which current flow is deactivated and the material is carried downstream.

[0049] Figure 2A illustrates an embodiment of the invention that includes a hybrid vacuum-dielectrophoretic injection scheme. As shown, microfluidic device **200** includes a cross microchannel configuration similar to the one depicted in Figure 1A in which one of the microchannels includes series of equidistantly spaced metal electrodes **202**. As further shown, electrical control system **204** is switchable between AC and DC pulses (e.g., via an XOR connection or the like) to effect dispersion-controlled material transport. Figure 2A also includes schematic current plot **206**, which depicts two cycles **208** of current flow from electrical control system **204** through the microchannel that includes series of equidistantly spaced metal capture electrodes **202**. Each cycle includes a period of AC flow **210** and a period of DC flow **212**. When AC is flowed between field electrode portions **214** and **216**, material is dielectrophoretically-captured at one or more of series of equidistantly spaced metal capture electrodes **202**, whereas upon switching to DC, the material is released from the potential wells at the capture electrodes and permitted to flow downstream.

[0050] Figure 2B, which depicts a magnified view of the intersection of microchannels from Figure 2A, illustrates one embodiment of a method of injecting discrete samples of material into microchannels for subsequent dispersion-controlled material transport and/or for other processing or analysis. The arrows indicate the direction of fluid flow in the depicted microchannel segments. In first step **218**, material is flowed into main microchannel **226** from intersecting side microchannel **228**, where the material flow stream is pinched or focused in the intersection by converging fluid flows (e.g., buffers, solvents, etc.) in main microchannel **226**. While material flow is pinched, second step **220** includes dielectrophoretically-capturing a discrete sample of material at first capture electrode **230** by flowing AC through main microchannel **226** as described above. Typically, first step **218** and second step **220** are performed approximately at the time (i.e., the material stream is pinched at about the same time AC is applied). Third step **222** generally includes withdrawing the material flow stream from main microchannel **226** back into side microchannel **228**, while maintaining AC flow

such that the discrete sample of material remains dielectrophoretically-captured at first capture electrode **230**. Thereafter, fourth step **224** includes, e.g., translocating the discrete sample of material to second capture electrode **232** by deactivating AC during flow (e.g., pressure-based flow) in main microchannel **226** and subsequently reactivating AC in the cyclic manner described above. In lieu of translocating the discrete sample, other processing steps such nucleic acid amplification, genotyping, or the like are optionally performed while the sample is captured at first capture electrode **230** or any other capture electrode. Other options include introducing multiple discrete samples of selected materials by performing repeated cycles of these injection methods followed by, e.g., selected processing steps.

[0051] According to the methods described herein, the relative drag and dielectrophoretic forces experienced by materials, such as polynucleotides are optionally adjusted, e.g., by attaching each material component to at least one separate microbead (e.g., a microbead made of a polar or polarizable material having a diameter greater than about 20 nm). For example, Figure 1D schematically depicts microbead **144** with attached polynucleotide **146**. This method of altering dielectrophoretic properties is effective, at least in part, because the fluid drag is proportional to the radius of the particle, while the dielectrophoretic force is proportional to the cube of the radius. Equations for estimating fluid drag and dielectrophoretic forces are described above. This method of adjusting dielectrophoretic properties generally provides greater flexibility over the control of dispersion during fluid transport. Methods of attaching materials to microbeads are widely known in the art and suitable microbeads are available from many different commercial suppliers including, e.g., Sperotech, Inc. (sperotech.com), Flow Cytometry Standards Corporation (fcstd.com), Sigma-Aldrich Corporation (sigma-aldrich.com), or the like.

[0052] The methods generally include capturing multiple discrete samples of the material in which each discrete sample is captured in a separate portion of the microchannel in which each separate portion is capable of generating a separate localized electric field. In these methods, at least two of the multiple captured discrete samples optionally include different constituent materials. In certain embodiments, a portion of the microfluidic device comprises a plurality of parallel microchannels and the methods

optionally include capturing the multiple discrete samples of the material in separate portions of each of the plurality of parallel microchannels in which each of the separate portions is capable of generating an independent localized electric field. For example, the plurality of parallel microchannels typically includes at least about 6, 12, 24, 48, 96, or more parallel microchannels. In addition, the methods optionally include assaying the multiple discrete samples for one or more detectable properties in each of the plurality of parallel microchannels simultaneously using a detector disposed in or proximal to the plurality of parallel microchannels.

[0053] During capture of materials in localized electric fields many different processes are optionally performed. For example, when each of the multiple captured discrete samples include a plurality of captured first nucleic acids, the methods optionally include flowing a set of second nucleic acids, e.g., under pressure into the microchannel, hybridizing the set of second nucleic acids to the plurality of captured first nucleic acids to provide a set of hybridized nucleic acids, and elongating the set of hybridized nucleic acids (e.g., with at least one thermostable polymerase, reverse transcriptase, or ligase). Optionally, at least two of the multiple captured discrete samples are different captured first nucleic acids. The flowing step optionally includes flowing one or more sets of molecular beacons along with the set of second nucleic acids in which the one or more sets of molecular beacons hybridize to the plurality of captured first nucleic acids or to the set of second nucleic acids to produce a detectable signal. The plurality of captured first nucleic acids and the set of second nucleic acids each independently include, e.g., primer nucleic acids, DNAs, RNAs, gDNAs, cDNAs, mtDNAs, mRNAs, tRNAs, rRNAs, snRNAs, or the like.

[0054] These steps, which typically occur in a nucleic acid amplification process (e.g., a polymerase chain reaction, a ligase chain reaction, or the like) or in a nucleic acid sequencing process, also optionally include denaturing the set of hybridized nucleic acids to provide a set of denatured nucleic acids, rehybridizing the set of denatured nucleic acids to provide a set of further hybridized nucleic acids, and extending the set of further hybridized nucleic acids (e.g., with at least one thermostable polymerase, reverse transcriptase, or ligase). A further option includes repeating the denaturing, rehybridizing, and extending steps at least once. The methods generally

include controlling temperature within the microchannel by using at least one selectable heating technique (e.g., joule heating, a hot plate, or the like). Joule heating is described in greater detail below. The methods also typically include flowing (i.e., in the second flowing step) the extended set of further hybridized nucleic acids from the microchannel into at least one other cavity of the microfluidic device using a fluid pressure force modulator or the like. Additionally, dispersion of the extended set of further hybridized nucleic acids is optionally controlled during the second flowing step. Furthermore, subsequent to at least one of the denaturing or repeated denaturing steps, a set of molecular beacons is optionally hybridized to the set of denatured nucleic acids to produce at least one detectable signal. Thereafter, the detectable signal is generally detected. Nucleic acid amplification and sequencing are discussed further below.

[0055] When materials (e.g., multiple discrete samples) are captured in localized electric fields many other assays or processes are also optionally performed. For example, the methods optionally include flowing at least one additional material through the microchannel that includes the multiple captured discrete samples of the material. The methods generally include detecting a detectable signal produced by one or more interactions of the additional material with one or more of the multiple captured discrete samples of the material. For example, the methods optionally include comparing the detectable signal with at least one standard. In certain embodiments, the methods also include separating one or more of the materials, the additional materials, or products of the one or more interactions. Typically, the material and the additional material each include one or more labels (e.g., a donor molecule, an acceptor molecule, a fluorophore, a chromophore, or the like) in which the one or more labels are identical to or different from one another.

[0056] Many different materials are optionally flowed in the devices and/or captured in the localized electric fields of the present invention. For example, the material and the additional material each independently optionally include, e.g., a cell, a set of cells, a microbead, a set of microbeads, a functionalized microbead, a set of functionalized microbeads, a reagent, a set of reagents, an atom, a set of atoms, a molecule, a set of molecules, a nucleic acid primer, a set of nucleic acid primers, a nucleic acid, a set nucleic acids, a neurotransmitter, a set of neurotransmitters, an antigen,

a set of antigens, a protein, a set of proteins, a peptide, a set of peptides, a lipid, a set of lipids, a carbohydrate, a set of carbohydrates, an organic molecule, a set of organic molecules, an inorganic molecule, a set of inorganic molecules, a drug, a set of drugs, a receptor ligand, a set of receptor ligands, an antibody, a set of antibodies, a cytokine, a set of cytokines, a chemokine, a set of chemokines, a hormone, or a set of hormones.

CAPTURE ELECTRODE CONFIGURATIONS

[0057] The methods and devices of the present invention utilize various capture electrode configurations to generate the localized electrical fields in which samples of polar or polarizable materials are captured to control dispersion, to perform assorted processes/assays, or the like. In general, capture electrode configurations of any arrangement, size, and/or shape consistent with the objectives of the invention are optionally included. In certain embodiments, capture electrode configurations include one or more capture electrodes disposed, e.g., between field electrodes, while in others capture electrode configurations include, e.g., pairs of opposing capture electrodes. Additionally, in some embodiments, capture electrodes are directly connected to external electrical power sources, while in others the connection is only indirect, e.g., via current flowing through a fluid-filled microchannel. Furthermore, in alternative embodiments external power supplies deliver AC and/or DC to produce localized electric fields. These and other aspects are described as follows.

[0058] The capture electrode configurations of the invention include many different arrangements. For example, in certain embodiments, capture electrode configurations include at least one capture electrode disposed between a first field electrode portion and a second field electrode portion in which at least a segment of the at least one capture electrode and the first and second field electrode portions are partially disposed within the microchannel. The electrical control system is typically operably connected to the first and second field electrode portions to permit current to flow through fluid disposed between the first and second field electrode portions in the microchannel to generate the localized electric fields. Figures 1A and 2A illustrate embodiments of this capture electrode configuration. In addition, capture electrodes generally have higher conductivities than fluids (e.g., buffers, solvents, electrolytes, or the like) that include the materials in the microchannel. Capture electrodes and the first

and second field electrode portions are typically fabricated from at least one metallic substance (e.g., Au, Pt, alloys, or the like).

[0059] In preferred embodiments, capture electrode configurations include multiple capture electrodes in which each capture electrode is disposed at least partially in or proximal to a different portion of the microchannel and is capable of generating a separate localized electric field. Specifically, the capture electrode configuration typically includes at least about 2, 3, 4, 5, 10, 15, 25, 50, 100, 500, 1000, or more separate capture electrodes. Optionally, each of the multiple capture electrodes is regularly or irregularly spaced from one another along a length of the microchannel. Regularly spaced capture electrode are depicted in, e.g., Figure 1A (i.e., series of equidistantly spaced metal capture electrodes **102**). For example, each of the multiple capture electrodes is optionally disposed at least about one μm (e.g., about 5 μm, 10 μm, 15 μm, 20 μm, 25 μm, or more) from one another along a length of the microchannel.

[0060] In other embodiments, the capture electrode configuration includes at least one capture electrode pair. Certain embodiments of these configurations are schematically depicted in Figure 3. For example, in one embodiment, capture electrode pair **300** optionally includes first capture electrode **302** disposed partially in or proximal to a top portion of the microchannel and second capture electrode **304** disposed partially in or proximal to a bottom portion of the microchannel in which the top and bottom portions oppose each other in the microchannel. (FIG. 3A). Alternatively, capture electrode pair **306** includes first capture electrode **308** disposed partially in or proximal to a first side portion of the microchannel and second capture electrode **310** disposed partially in or proximal to a second side portion of the microchannel in which the first and second side portions oppose each other in the microchannel. (FIG. 3B). As a further alternative, capture electrode pair **312** includes first capture electrode **314** and second capture electrode **316**, both disposed in or proximal to a bottom portion of the microchannel. (FIG. 3C). Optionally, both capture electrodes of a pair are both disposed in or proximal to a top or side portion of the microchannel. When multiple capture electrode pairs are provided in the same device, any combination of the configurations depicted in Figure 3 are optionally included. Typically, at least one segment of each electrode in the capture electrode pair substantially oppose each other in the

microchannel. Additionally, each electrode of a capture electrode pair is generally directly connected to an electrical control system, e.g., for selectively flowing AC and/or DC to electrode pairs for generating localized electric fields.

[0061] When a potential difference is applied across the electrodes in a capture electrode pair, a localized electric field is generated between the capture electrodes of the pair. The strength of the localized electric field will typically be largest where the separation between the capture electrodes is smallest. Polar or polarizable materials will aggregate in these localized electric fields, e.g., during pressure-based flow of the materials through a microchannel that includes the capture electrode pairs.

[0062] As schematically illustrated in Figure 3, lines of force **318** of the localized electric field at each capture electrode pair are directed substantially vertically or substantially horizontally in the portion of the microchannel. As used herein, a "vertically" directed line of force is substantially directed, in either direction, along or proximal to a plane that extends, in one dimension, from the highest to the lowest internal surface of a microchannel (e.g., along the length of a cross-sectional line or axis of the microchannel), when the microfluidic device is oriented for typical operational usage. A vertical line of force is oriented approximately 90° from where a horizontal line of force (discussed below) would be oriented. *See, e.g.,* Figures 3A and 4. A "horizontally" directed line of force, as used herein, is directed substantially, in either direction, along or proximal to a plane that extends, in one dimension, from one substantially vertical internal microchannel surface to another (e.g., substantially parallel to the dimension of the lowest internal microchannel surface that extends from one vertical internal surface to another), when the microfluidic device is oriented for typical operational usage. A horizontal line of force is oriented approximately 90° from where a vertical line of force (discussed above) would be oriented. *See, e.g.,* Figures 3B and 3C.

[0063] The capture electrode pairs of the present invention include various embodiments. For example, individual electrodes in capture electrode pairs optionally include shapes independently selected from, e.g., regular n-sided polygons, irregular n-sided polygons, triangles, squares, rectangles, trapezoids, circles, ovals, or the like. In addition, individual electrodes in capture electrode pairs are typically separated from each another by at least about one μm. In certain embodiments, individual electrodes in

capture electrode pairs include opposing electrically conductive wires disposed at angles relative to one another in the range of from about 0° to about 90°. Optionally, individual electrodes in capture electrode pairs independently include, e.g., an electrically conductive wire, an electrically conductive coating, an electrically conductive plate, or the like. For example, individual electrodes in capture electrode pairs optionally include a surface area that ranges from about one μm^2 to about an entire internal surface of the microchannel.

[0064] In certain preferred embodiments, the capture electrode configuration includes multiple capture electrode pairs in which each electrode of each capture electrode pair is disposed at least partially in or proximal to a different portion of the microchannel. For example, the capture electrode configuration optionally includes at least about 2, 3, 4, 5, 10, 15, 25, 50, 100, 500, 1000, or more separate capture electrode pairs. Each of the multiple capture electrode pairs is capable of generating a separate localized electric field. Additionally, each of the multiple capture electrode pairs is regularly or irregularly spaced from one another along a length of the microchannel. For example, each of the multiple capture electrode pairs is optionally disposed at least about one μm (e.g., about 5 μm, 10 μm, 15 μm, 20 μm, 25 μm, or more) from one another along a length of the microchannel. Furthermore, individual localized electric fields generated by the capture electrode pairs are each independently activated or deactivated.

[0065] To further illustrate, linear (e.g., Figure 5A) and two-dimensional (e.g., Figure 5B) arrays of capture electrode pairs are optionally fabricated when, e.g., the capture electrodes (e.g., in the form of electrically conductive wires) are no longer parallel, but skew (i.e., not intersecting, but with variable separation). As schematically shown in Figure 5, which includes top views of two microchannels, capture electrode pairs (highlighted in ovals) are produced when one capture electrode of each pair (e.g., a first wire) is disposed in or proximal to the top of the microchannel and the other capture electrode of each pair (e.g., a second wire) is disposed in or proximal to the bottom of the microchannel. These multiple capture electrode pairs are optionally either activated or energized simultaneously, or individually if multiple conductive wires are utilized., e.g., to capture samples of material at selected capture electrode pairs.

AMPLIFICATION AND SEQUENCING METHODS

[0066] The amplification and sequencing of nucleic acids are of fundamental importance to many disciplines, including medical diagnostics, molecular biology, forensics, genomics, and molecular evolution. Both techniques are optionally adapted to the high-throughput serial or parallel devices and methods of the present invention. For example, nucleic acid amplification methods, such as the widely-known polymerase chain reaction (PCR) typically include repeated thermocycles that denature double-stranded target nucleic acids, hybridize single-stranded target and primer nucleic acids, and elongate primer strands with a polymerase. Many variations of this basic technique are also known, including asymmetric PCRs, assembly PCRs, reverse transcription PCRs (RT-PCR), ligase chain reactions (LCR), or the like. These amplification methods are described further in, e.g., F.M. Ausubel et al., eds., Current Protocols in Molecular Biology, Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (supplemented through 2000), Sambrook et al., Molecular Cloning - A Laboratory Manual (2nd Ed.), Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989, Newton and Graham, PCR (2nd Ed.), Scientific Publishers, Oxford, England, 1997, Herrington and O'Leary, eds., PCR 3: In Situ Hybridization, IRL Press at Oxford University Press, Inc., Oxford, England, 1997, Smeltzer, ed., PCR In Bioanalysis: Methods in Molecular Biology, Vol. 92, Humana Press, Towata, N.J., 1998, and Innis et al., eds., PCR Strategies, Academic Press, San Diego, CA, 1995.

[0067] Nucleic acid sequencing or genotyping techniques, such as the Sanger dideoxy method, which is generally known in the art, are also optionally performed using the devices and methods of the present invention. The dideoxy method typically includes performing repeated thermocycles to extend primers hybridized to single-stranded target nucleic acids with a polymerase (e.g., Taq DNA polymerase) in reaction mixtures that also include the four dNTPs, and one of the four dideoxy analogues (e.g., a 2',3'-dideoxy analog), which terminates the extension reaction upon incorporation. Sequences are typically determined by detecting signals, e.g., from radioactive or fluorescent labels incorporated into the extended primer strands. Sequencing formats, including cycle sequencing are described in a variety of references,

including, e.g., Rapley, PCR Sequencing Protocols, Humana Press, Towata, N.J., 1996, Griffin and Griffin, Eds., DNA Sequencing Protocols, Humana Press, Towata, N.J., 1993, Roe et al., DNA Isolation and Sequencing, John Wiley & Sons, New York, N.Y., 1996, Adams et al., Eds., Automated DNA Sequencing and Analysis, Academic Press, San Diego, CA, 1994, and Alphey, DNA Sequencing from Experimental Methods to Bioinformatics, Springer Verlag, New York, N.Y., 1997. *See also*, Maxam, A.M. and Gilbert, W. (1977) "A New Method for Sequencing DNA," Proc. Natl. Acad. Sci. U.S.A. 74:560-564, Sanger, F. *et al.* (1977) "DNA Sequencing with Chain-Terminating Inhibitors," Proc. Natl. Acad. Sci. U.S.A. 74:5463-5467, Hunkapiller, T. *et al.* (1991) "Large-Scale and Automated DNA Sequence Determination," Science 254:59-67, and Pease, A.C. *et al.* (1994) "Light-Generated Oligonucleotide Arrays for Rapid DNA Sequence Analysis," Proc. Natl. Acad. Sci. U.S.A. 91:5022-5026. Applications of sequencing in microfluidic systems are found in, e.g., published PCT applications WO 98/45481, WO 00/50172, and WO 00/50642.

[0068] The present invention provides improved high-throughput in-line methods and devices for performing nucleic acid amplification and/or sequencing. Figure 6A illustrates an embodiment of the invention that is optionally utilized to "parallelize" in-line nucleic acid amplification and/or sequencing. As shown, microfluidic device **600** includes set of parallelized microchannels **602**, each of which includes series of equidistantly spaced metal electrodes **604**. As further shown, electrical control system **606** is switchable between AC and DC pulses (e.g., via an XOR connection or the like) to effect dispersion-controlled material transport (e.g., when utilized in conjunction with pressure-based fluid movement) and/or to effect, e.g., nucleic acid capture at one or more of series of equidistantly spaced metal electrodes **604**. Although not shown, a three well vacuum pumping system for applying pressure-based flow is also optionally operably connected to the device via wells **608**, **610**, and **612**. In addition, two of the wells (namely, **608** and **612**) include field electrode portions (**614** and **616**, respectively) for the application of current from electrical control system **606**, e.g., to effect material capture in localized electric fields. As described above, when AC is flowed between field electrode portions **614** and **616**, polar or polarizable materials (e.g., nucleic acids) are dielectrophoretically-captured at one or more of series of equidistantly

spaced metal capture electrodes **604**, whereas upon switching to DC, the material is released from the potential wells at the capture electrodes and permitted to flow downstream. Note, that a capture electrode configuration that includes capture electrode pairs, as described above, is also optionally utilized, e.g., in lieu of or with the capture electrode configuration depicted in Figure 6A.

[0069] The nucleic acid amplification methods of the present invention include positioning a set of first nucleic acids in a portion of a microchannel (e.g., at one or more of series of equidistantly spaced metal capture electrodes **604**), flowing a set of second nucleic acids, e.g., under pressure, into the microchannel (e.g., from well **608**), and capturing the set of first nucleic acids in localized electric fields generated at one or more of series of equidistantly spaced metal capture electrodes **604** in the microchannel. (FIG. 6A). The methods also include hybridizing the set of second nucleic acids to the set of captured first nucleic acids to provide a set of hybridized nucleic acids and elongating the set of hybridized nucleic acids (e.g., with a thermostable polymerase, a reverse transcriptase, or a ligase) to amplify the nucleic acids. These steps typically occur in one or more polymerase chain reactions (e.g., one or more asymmetric PCRs, assembly PCRs, RT-PCRs, LCRs, or the like).

[0070] The methods optionally include positioning at least two sets of first nucleic acids in which each set includes different first nucleic acids. Figure 6B schematically depicts nucleic acids embedded in meltable agarose gel **618** positioned on a magnified view of capture electrode **620** from series of equidistantly spaced metal capture electrodes **604**. Additionally, the flowing step optionally includes flowing one or more sets of molecular beacons along with the set of second nucleic acids in which the one or more sets of molecular beacons hybridize to the set of captured first nucleic acids or to the set of second nucleic acids to produce at least one detectable signal. Other known methods for assessing the progress of thermocycling reactions are also optionally utilized. Furthermore, the set of captured first nucleic acids and the set of second nucleic acids each independently include, e.g., primer nucleic acids, DNAs, RNAs, gDNAs, cDNAs, mtDNAs, mRNAs, tRNAs, rRNAs, snRNAs, or the like.

[0071] In one embodiment, positioning step **622** includes disposing meltable gel embedded first nucleic acids at one or more of series of equidistantly spaced

metal capture electrodes **604**. (FIG. 6C). Thereafter, capturing steps **624** and **626** include activating a plurality of localized electric fields in which each localized electric field corresponds to at least one of the multiple portions (i.e., capture electrodes) of the microchannel that includes the meltable gel embedded first nucleic acids such that the localized electric fields melt the meltable gel and capture the first nucleic acids embedded therein. Optionally, nucleic acids (e.g., primers, target sequences, or the like), enzymes, or the like are positioned at selected capture electrodes according to the dispersion-controlled transport methods described above.

[0072] The methods of amplifying nucleic acids optionally further include denaturing the set of hybridized nucleic acids to provide a set of denatured nucleic acids, rehybridizing the set of denatured nucleic acids to provide a set of further hybridized nucleic acids, and extending (e.g., with at least one thermostable polymerase, reverse transcriptase, or ligase) the set of further hybridized nucleic acids to further amplifying the set of nucleic acids. Optionally, the denaturing, rehybridizing, and extending steps are repeated at least once. The methods generally include a second flowing step that includes flowing the extended set of further hybridized nucleic acids from the microchannel into at least one other cavity of the microfluidic device using a fluid pressure force modulator or the like. Dispersion of the extended set of further hybridized nucleic acids is typically controlled during the second flowing step. As an additional option, subsequent to at least one of the denaturing or repeated denaturing steps, at least one set of molecular beacons is hybridized to the set of denatured nucleic acids to produce a detectable signal. Thereafter, the methods typically include detecting the detectable signal.

[0073] The methods typically include controlling temperature within the microchannel by using at least one selectable heating technique (e.g., joule heating, a hot plate, or the like). Joule heating is typically produced by flowing current through an electrode or other conductive component positioned within a well, microscale channel, or other cavity within the device. The resulting flow of current into fluid within the well, channel, or cavity results in resistive heating of the fluid (i.e., by dissipating energy through the electrical resistance of the fluid). By substantially increasing the current across the channel, rapid temperature changes are induced that are optionally monitored

by conductivity. Because nanoliter volumes of fluid have tiny thermal mass, transitions between temperatures are typically extremely short. For example, oscillations between any two temperatures above 0°C and below 100°C in 100 milliseconds have been performed. Thus, the present invention optionally uses power sources that pass electrical current through microchannels or other devices cavities for heating purposes. In exemplary embodiments, fluid passes through a microchannel of a desired cross-section (e.g., diameter) to enhance thermal transfer of energy from the current to the fluid.

[0074] To selectively control the temperature of fluid in a region of a channel, a power supply applies voltage and/or current in various ways. For instance, a power supply optionally applies DC (e.g., to selected capture electrode pairs of a capture electrode configuration) or AC, which passes through the microchannel and into a microchannel region which is, e.g., smaller in cross-section to heat fluid in the region. Alternatively, a power supply applies a pulse or impulse of current and/or voltage, which passes through the microchannel and into a channel region to heat fluid in the region. Pulse width, shape, and/or intensity are optionally adjusted, e.g., to heat the fluid substantially while moving the fluid. Still further, the power supply optionally applies any combination of DC, AC, and pulse, depending upon the application. In practice, direct application of electric current to fluids in the microchannels of the invention results in extremely rapid and easily controlled changes in temperature.

[0075] A controller or computer such as a personal computer is generally used to monitor the temperature of the fluid in the region of the channel where the fluid is heated. The controller or computer typically receives current and voltage information from, e.g., the power supply and identifies or detects fluid temperature in the channel region. The controller or computer also typically receives current information from an operably connected detector, e.g., when a selected particle is detected, which triggers the flow of current through, e.g., one or more joule heating electrodes. Depending upon the desired temperature of fluid in the region, the controller or computer adjusts voltage and/or current to meet the desired fluid temperature. Controllers and computers are discussed further below. Joule heating is also described further in, e.g., in 08/977,528, entitled "Electrical Current for Controlling Fluid Temperatures in Microchannels," filed November 25, 1997 by Calvin Chow, Anne R. Kopf-Sill and J. Wallace Parce, and in

WO 98/45481, entitled "Closed-Loop Biochemical Analyzers," filed April 3, 1998, by Knapp et al.

[0076] There are various other techniques that are also optionally used to heat fluidic materials within microchannels of the devices of the present invention. For example, resistive heating typically results from current applied to conductively coated well or microchannel portions, from one or more electrodes directly (e.g., a thermocouple, etc.), or the like. Other heating methods optionally include directing, e.g., light from a laser source through one or more fiber optic cables to, e.g., wells that fluidly communicate with analysis channels. Heat from other external sources is also optionally utilized in the methods described herein.

[0077] As mentioned, following amplification or sequencing reactions, reaction products, such as the amplified target sequences (i.e., amplicons) are typically subjected to additional downstream processing. For example, reaction products are typically flowed, e.g., to other regions of the microfluidic device under dispersion-controlled fluid transport, as described herein, for optional separation, detection (e.g., of labeled dideoxy reaction products to obtain sequencing information, etc.), and/or other processing. Detection systems are described further below.

MICROFLUIDIC DEVICES

[0078] Many different microscale systems are optionally adapted for use in the dispersion-controlled transport and processing methods of the present invention. These systems are described in numerous publications by the inventors and their coworkers, including certain issued U.S. Patents, such as U.S. Patent Nos. 5,699,157 (J. Wallace Parce) issued 12/16/97, 5,779,868 (J. Wallace Parce et al.) issued 07/14/98, 5,800,690 (Calvin Y.H. Chow et al.) issued 09/01/98, 5,842,787 (Anne R. Kopf-Sill et al.) issued 12/01/98, 5,852,495 (J. Wallace Parce) issued 12/22/98, 5,869,004 (J. Wallace Parce et al.) issued 02/09/99, 5,876,675 (Colin B. Kennedy) issued 03/02/99, 5,880,071 (J. Wallace Parce et al.) issued 03/09/99, 5,882,465 (Richard J. McReynolds) issued 03/16/99, 5,885,470 (J. Wallace Parce et al.) issued 03/23/99, 5,942,443 (J. Wallace Parce et al.) issued 08/24/99, 5,948,227 (Robert S. Dubrow) issued 09/07/99, 5,955,028 (Calvin Y.H. Chow) issued 09/21/99, 5,957,579 (Anne R. Kopf-Sill et al.) issued 09/28/99, 5,958,203 (J. Wallace Parce et al.) issued 09/28/99, 5,958,694 (Theo T.

Nikiforov) issued 09/28/99, 5,959,291 (Morten J. Jensen) issued 09/28/99, 5,964,995 (Theo T. Nikiforov et al.) issued 10/12/99, 5,965,001 (Calvin Y. H. Chow et al.) issued 10/12/99, 5,965,410 (Calvin Y. H. Chow et al.) issued 10/12/99, 5,972,187 (J. Wallace Parce et al.) issued 10/26/99, 5,976,336 (Robert S. Dubrow et al.) issued 11/2/99, 5,989,402 (Calvin Y. H. Chow et al.) issued 11/23/99, 6,001,231 (Anne R. Kopf-Sill) issued 12/14/99, 6,011,252 (Morten J. Jensen) issued 1/4/00, 6,012,902 (J. Wallace Parce) issued 1/11/00, 6,042,709 (J. Wallace Parce et al.) issued 3/28/00, 6,042,710 (Robert S. Dubrow) issued 3/28/00, 6,046,056 (J. Wallace Parce et al.) issued 4/4/00, 6,048,498 (Colin B. Kennedy) issued 4/11/00, 6,068,752 (Robert S. Dubrow et al.) issued 5/30/00, 6,071,478 (Calvin Y. H. Chow) issued 6/6/00, 6,074,725 (Colin B. Kennedy) issued 6/13/00, 6,080,295 (J. Wallace Parce et al.) issued 6/27/00, 6,086,740 (Colin B. Kennedy) issued 7/11/00, 6,086,825 (Steven A. Sundberg et al.) issued 7/11/00, 6,090,251 (Steven A. Sundberg et al.) issued 7/18/00, 6,100,541 (Robert Nagle et al.) issued 8/8/00, 6,107,044 (Theo T. Nikiforov) issued 8/22/00, 6,123,798 (Khushroo Gandhi et al.) issued 9/26/00, 6,129,826 (Theo T. Nikiforov et al.) issued 10/10/00, 6,132,685 (Joseph E. Kersco et al.) issued 10/17/00, 6,148,508 (Jeffrey A. Wolk) issued 11/21/00, 6,149,787 (Andrea W. Chow et al.) issued 11/21/00, 6,149,870 (J. Wallace Parce et al.) issued 11/21/00, 6,150,119 (Anne R. Kopf-Sill et al.) issued 11/21/00, 6,150,180 (J. Wallace Parce et al.) issued 11/21/00, 6,153,073 (Robert S. Dubrow et al.) issued 11/28/00, 6,156,181 (J. Wallace Parce et al.) issued 12/5/00, 6,167,910 (Calvin Y. H. Chow) issued 1/2/01, 6,171,067 (J. Wallace Parce) issued 1/9/01, 6,171,850 (Robert Nagle et al.) issued 1/9/01, 6,172,353 (Morten J. Jensen) issued 1/9/01, 6,174,675 (Calvin Y. H. Chow et al.) issued 1/16/01, 6,182,733 (Richard J. McReynolds) issued 2/6/01, 6,186,660 (Anne R. Kopf-Sill et al.) issued 2/13/01, 6,221,226 (Anne R. Kopf-Sill) issued April 24, 2001, 6,233,048 (J. Wallace Parce) issued May 15, 2001, 6,235,175 (Robert S. Dubrow et al.) issued 5/22/01, 6,235,471 (Michael Knapp et al.) issued 5/22/01, 6,238,538 (J. Wallace Parce et al.) issued 5/29/01, 6,251,343 (Robert S. Dubrow et al.) issued 6/26/01, 6,267,858 (J. Wallace Parce et al.) issued 7/31/01, 6,274,089 (Andrea W. Chow et al.) issued 8/14/01, 6,274,337 (J. Wallace Parce et al.) issued 8/14/01, 6,287,520 (J. Wallace Parce et al.) issued 9/11/01, 6,287,774 (Theo T. Nikiforov) issued 9/11/01, 6,303,343 (Anne R. Kopf-Sill) issued 10/16/01, 6,306,590

(Tammy Burd Mehta et al.) issued 10/23/01, and 6,306,659 (J. Wallace Parce et al.) issued 10/23/01.

[0079] Systems adapted for use with the devices of the present invention are also described in, e.g., various published PCT applications, including WO 98/00231, WO 98/00705, WO 98/00707, WO 98/02728, WO 98/05424, WO 98/22811, WO 98/45481, WO 98/45929, WO 98/46438, and WO 98/49548, WO 98/55852, WO 98/56505, WO 98/56956, WO 99/00649, WO 99/10735, WO 99/12016, WO 99/16162, WO 99/19056, WO 99/19516, WO 99/29497, WO 99/31495, WO 99/34205, WO 99/43432, WO 99/44217, WO 99/56954, WO 99/64836, WO 99/64840, WO 99/64848, WO 99/67639, WO 00/07026, WO 00/09753, WO 00/10015, WO 00/21666, WO 00/22424, WO 00/26657, WO 00/42212, WO 00/43766, WO 00/45172, WO 00/46594, WO 00/50172, WO 00/50642, WO 00/58719, WO 00/60108, WO 00/70080, WO 00/70353, WO 00/72016, WO 00/73799, WO 00/78454, WO 01/02850, WO 01/14865, WO 01/17797, and WO 01/27253.

[0080] The methods of the invention are generally performed within fluidic channels along which reagents, enzymes, samples, buffers, and other fluids are disposed and/or flowed. In some cases, the channels are simply present in a capillary or pipettor element, e.g., a glass, fused silica, quartz or plastic capillary. The capillary element is fluidly coupled to a source of, e.g., the reagent, sample, modulator, or other solution (e.g., by dipping the capillary element into a well on a microtiter plate), which is then flowed along the channel (e.g., a microchannel) of the element. In preferred embodiments, the capillary element is integrated into the body structure of a microfluidic device. The term "microfluidic," as used herein, generally refers to one or more fluid passages, chambers or conduits which have at least one internal cross-sectional dimension, e.g., depth, width, length, diameter, etc., that is less than 500μm, and typically between about 0.1 μm and about 500 μm.

[0081] In the devices of the present invention, the microscale channels or cavities typically have at least one cross-sectional dimension between about 0.1 μm and 200 μm, preferably between about 0.1 μm and 100 μm, and often between about 0.1 μm and 50 μm. Accordingly, the microfluidic devices or systems prepared in accordance with the present invention typically include at least one microscale channel, usually at least two

intersecting microscale channels, and often, three or more intersecting channels disposed within a single body structure. Channel intersections may exist in a number of formats, including cross intersections, "Y" and/or "T" intersections, or any number of other structures whereby two channels are in fluid communication.

[0082] The body structures of the microfluidic devices described herein are typically manufactured from two or more separate portions or substrates which when appropriately mated or joined together, form the microfluidic device of the invention, e.g., containing the channels and/or chambers described herein. During body structure fabrication, the microfluidic devices described herein will typically include a top portion, a bottom portion, and an interior portion, wherein the interior portion substantially defines the channels and chambers of the device. Typically, capture electrode configurations are disposed between substrates during fabrication such that when completed, the devices include, e.g., capture electrodes in or proximal to selected regions (e.g., microchannels or other cavities) of the devices.

[0083] In one aspect, a bottom portion of the unfinished device includes a solid substrate that is substantially planar in structure, and which has at least one substantially flat upper surface. Channels are typically fabricated on one surface of the device and sealed by overlaying the channels with an upper substrate layer. A variety of substrate materials are optionally employed as the upper or bottom portion of the device. Typically, because the devices are microfabricated, substrate materials will be selected based upon their compatibility with known microfabrication techniques, e.g., photolithography, wet chemical etching, laser ablation, air abrasion techniques, LIGA, reactive ion etching, injection molding, embossing, and other techniques. The substrate materials are also generally selected for their compatibility with the full range of conditions to which the microfluidic devices may be exposed, including extremes of pH, temperature, electrolyte concentration, and/or other properties. Accordingly, in some preferred aspects, the substrate material may include materials normally associated with the semiconductor industry in which such microfabrication techniques are regularly employed, including, e.g., silica-based substrates, such as glass, quartz, silicon or polysilicon, as well as other substrate materials, such as gallium arsenide and the like. In the case of semiconductive materials, it will often be desirable to provide an insulating coating or layer, e.g., silicon

oxide, over the substrate material, and particularly in those applications where electric fields are to be applied to the device or its contents.

[0084] In additional preferred aspects, the substrate materials will comprise polymeric materials, e.g., plastics, such as polymethylmethacrylate (PMMA), polycarbonate, polytetrafluoroethylene (TEFLON™), polyvinylchloride (PVC), polydimethylsiloxane (PDMS), polysulfone, polystyrene, polymethylpentene, polypropylene, polyethylene, polyvinylidine fluoride, ABS (acrylonitrile-butadiene-styrene copolymer), and the like. Such polymeric substrates are readily manufactured using available microfabrication techniques, as described above, or from microfabricated masters, using known molding techniques, such as injection molding, embossing or stamping, or by polymerizing the polymeric precursor material within the mold (*See*, e.g., U.S. Patent No. 5,512,131). Such polymeric substrate materials are preferred for their ease of manufacture, low cost and disposability, as well as their general inertness to most extreme reaction conditions. Again, these polymeric materials optionally include treated surfaces, e.g., derivatized or coated surfaces, to enhance their utility in the microfluidic system, e.g., to provide enhanced fluid direction, e.g., as described in U.S. Pat. No. 5,885,470 (J. Wallace Parce et al.) issued 3/23/99, and which is incorporated herein by reference in its entirety for all purposes.

[0085] The channels and/or cavities of the microfluidic devices are typically fabricated into the upper surface of the bottom substrate or portion of the device, as microscale grooves or indentations, using the above described microfabrication techniques. The top portion or substrate also comprises a first planar surface, and a second surface opposite the first planar surface. In the microfluidic devices prepared in accordance with certain aspects of the methods described herein, the top portion can include at least one aperture, hole or port disposed therethrough, e.g., from the first planar surface to the second surface opposite the first planar surface. In other embodiments, the port(s) are optionally omitted, e.g., where fluids are introduced solely through external capillary elements.

[0086] The first planar surface of the top portion or substrate is then mated, e.g., placed into contact with, and bonded to the planar surface of the bottom substrate, covering and sealing the grooves and/or indentations in the surface of the bottom substrate, to form the channels and/or chambers (i.e., the interior portion) of the device at the interface of these two components. Bonding of substrates is typically carried out by any of

a number of different methods, e.g., thermal bonding, solvent bonding, ultrasonic welding, and the like. The finished body structure of a device is a unitary structure that houses, e.g., the channels and/or chambers of the device. As mentioned, capture electrode configurations are typically disposed between substrates during fabrication such upon completion, the devices include, e.g., capture electrodes in or proximal to selected regions (e.g., microchannels or other cavities) of the devices.

[0087] The hole(s) in the top of the finished device is/are oriented to fluidly communicate with at least one of the channels and/or cavities. In the completed device, the hole(s) optionally function as reservoirs for facilitating fluid or material introduction into the channels or chambers of the device, as well as providing ports at which, e.g., pressure elements (e.g., vacuum sources, etc.) are optionally placed into contact with fluids within the device, allowing application of pressure gradients along the channels of the device to control and direct fluid transport within the device. In optional embodiments, extensions are provided over these reservoirs to allow for increased fluid volumes, permitting longer running assays, and better controlling fluid flow parameters, e.g., hydrostatic pressures. Examples of methods and apparatuses for providing such extensions are described in U.S. Application No. 09/028,965, filed February 24, 1998, and incorporated herein by reference. These devices are optionally coupled to a sample introduction port, e.g., a pipettor or capillary element, which serially introduces multiple samples, e.g., from the wells of a microtiter plate. Thus, in some embodiments, both reservoirs in the upper surface and external capillary elements are present in a single device.

[0088] The sources of reagents, enzymes, samples, buffers, and other materials are optionally fluidly coupled to the microchannels in any of a variety of ways. In particular, those systems comprising sources of materials set forth in Knapp et al. "Closed Loop Biochemical Analyzers" (WO 98/45481; PCT/US98/06723) and U.S. Pat. No. 5,942,443 issued August 24, 1999, entitled "High Throughput Screening Assay Systems in Microscale Fluidic Devices" to J. Wallace Parce et al. and, e.g., in 60/128,643 filed April 4, 1999, entitled "Manipulation of Microparticles In Microfluidic Systems," by Mehta et al. are applicable.

[0089] In these systems and as noted above, a capillary or pipettor element (i.e., an element in which components are optionally moved from a source to a microscale element such as a second channel or reservoir) is temporarily or permanently

coupled to a source of material. The source is optionally internal or external to a microfluidic device that includes the pipettor or capillary element. Example sources include microwell plates, membranes or other solid substrates comprising lyophilized components, wells or reservoirs in the body of the microscale device itself and others.

FLOW OF MATERIALS IN MICROFLUIDIC SYSTEMS

[0090] The flowing of reagents or other materials along the microchannels of the devices described herein is optionally carried out by a number of mechanisms, including pressure-based flow, electrokinetic flow, hydrodynamic flow, gravity-based flow, centripetal or centrifugal flow, or mechanisms that utilize a hybrid of these techniques. In a preferred aspect, a pressure differential is used to flow materials along the microchannels or other cavities of the devices described herein (e.g., at field electrodes, selected capture electrodes, or the like).

[0091] Application of a pressure differential along a channel is carried out by any of a number of approaches. For example, it may be desirable to provide relatively precise control of the flow rate of materials, e.g., to precisely control incubation or separation times, etc. As such, in many preferred aspects, flow systems that are more active than hydrostatic pressure driven systems are employed. In certain cases, materials may be flowed by applying a pressure differential across the length of an analysis channel (e.g., a microchannel that includes a capture electrode configuration). For example, a pressure source (positive or negative) is applied at the reagent or material reservoir at one end of the analysis channel, and the applied pressure forces the materials through the channel. The pressure source is optionally pneumatic, e.g., a pressurized gas, or a positive displacement mechanism, i.e., a plunger fitted into a reagent reservoir, for forcing the reagents through the analysis channel. Alternatively, a vacuum source is applied to a reservoir at the opposite end of the channel to draw the reagents through the channel. Pressure or vacuum sources may be supplied external to the device or system, e.g., external vacuum or pressure pumps sealably fitted to the inlet or outlet of the analysis channel, or they may be internal to the device, e.g., microfabricated pumps integrated into the device and operably linked to the analysis channel. Examples of microfabricated pumps have been widely described in the art. *See*, e.g., published International Application No. WO 97/02357.

[0092] In an alternative simple passive aspect, the materials are deposited in a reservoir or well at one end of an analysis channel and at a sufficient volume or depth, that the sample of material creates a hydrostatic pressure differential along the length of the analysis channel, e.g., by virtue of it having greater depth than a reservoir at an opposite terminus of the channel. The hydrostatic pressure then causes the materials to flow along the length of the channel. Typically, the reservoir volume is quite large in comparison to the volume or flow through rate of the channel, e.g., 10 μl reservoirs, vs. 1000 μm^2 channel cross-section. As such, over the time course of the assay/separation, the flow rate of the materials will remain substantially constant, as the volume of the reservoir, and thus, the hydrostatic pressure changes very slowly. Applied pressure is then readily varied to yield different material flow rates through the channel. In screening applications, varying the flow rate of the materials is optionally used to vary the incubation time of the materials. In particular, by slowing the flow rate along the channel, one can effectively lengthen the amount of time between introduction of materials and detection of a particular effect. Alternatively, analysis channel lengths, detection points, or material introduction points are varied in fabrication of the devices, to vary incubation times.

[0093] In further alternate aspects, hydrostatic, wicking and capillary forces are additionally, or alternately, used to provide for fluid flow. *See*, e.g., "Method and Apparatus for Continuous Liquid Flow in Microscale Channels Using Pressure Injection, Wicking and Electrokinetic Injection," by Alajoki et al., USSN 09/245,627, filed February 5, 1999. In these methods, an adsorbent material or branched capillary structure is placed in fluidic contact with a region where pressure is applied, thereby causing fluid to move towards the adsorbent material or branched capillary structure.

[0094] In alternative aspects, flow of materials is driven by inertial forces. In particular, the analysis channel is optionally disposed in a substrate that has the conformation of a rotor, with the analysis channel extending radially outward from the center of the rotor. The materials are deposited in a reservoir that is located at the interior portion of the rotor and is fluidly connected to the channel. During rotation of the rotor, the centripetal force on the materials forces the materials through the analysis channel, outward toward the edge of the rotor. Multiple analysis channels are optionally provided in the rotor to perform multiple different analyses. Detection of a detectable signal produced by the materials is then carried out by placing a detector under the spinning

rotor and detecting the signal as the analysis channel passes over the detector. Examples of rotor systems have been previously described for performing a number of different assay types. *See*, e.g., Published International Application No. WO 95/02189. Test compound reservoirs are optionally provided in the rotor, in fluid communication with the analysis channel, such that the rotation of the rotor also forces the test compounds into the analysis channel.

[0095] For purposes of illustration, the discussion has focused on a single channel and accessing capillary; however, it will be readily appreciated that these aspects may be provided as multiple parallel analysis channels (e.g., each including a capture electrode configuration) and accessing capillaries, in order to substantially increase the throughput of the system. Specifically, single body structures may be provided with multiple parallel analysis channels coupled to multiple sample accessing capillaries that are positioned to sample multiple samples at a time from sample libraries, e.g., multiwell plates. As such, these capillaries are generally spaced at regular distances that correspond with the spacing of wells in multiwell plates, e.g., 9 mm centers for 96 well plates, 4.5 mm for 384 well plates, and 2.25 mm for 1536 well plates.

[0096] In additional alternative aspects, flow of reagents is driven by inertial forces. In particular, the analysis channel is optionally disposed in a substrate that has the conformation of a rotor, with the analysis channel extending radially outward from the center of the rotor. The reagents are deposited in a reservoir that is located at the interior portion of the rotor and is fluidly connected to the channel. During rotation of the rotor, the centripetal force on the reagents forces the reagents through the analysis channel, outward toward the edge of the rotor. Multiple analysis channels are optionally provided in the rotor to perform multiple different analyses. Detection of a detectable signal produced by the reagents is then carried out by placing a detector under the spinning rotor and detecting the signal as the analysis channel passes over the detector. Examples of rotor systems have been previously described for performing a number of different assay types. See, e.g., Published International Application No. WO 95/02189. Test compound reservoirs are optionally provided in the rotor, in fluid communication with the analysis channel, such that the rotation of the rotor also forces the test compounds into the analysis channel.

DEVICES AND INTEGRATED SYSTEMS

[0097] The present invention also relates to a device or integrated system which is typically used to perform the high-throughput dispersion-controlled pressure-based material transport and other processes described herein. The microfluidic devices each typically include a substrate having a surface and at least one microchannel fabricated into the surface of the substrate in which the microchannel includes at least one capture electrode configuration. Capture electrode configurations are described in greater detail above. The device also includes a source of a material in fluid communication with the microchannel, an electrical control system (e.g., a selectable electrical field source, an alternating current source, a direct current source, and/or an arbitrary current source) operably connected to the at least one capture electrode configuration for generating at least one localized electric field in the at least one capture electrode configuration to reversibly capture the material, a pressure-based fluid direction system (e.g., a fluid pressure force modulator or the like) operably connected to the microfluidic device for inducing flow of the material in the microchannel, and a cover mated with the surface of the substrate. In certain embodiments, the at least one microchannel of the microfluidic device includes a plurality of parallel microchannels fabricated into the surface of the substrate in which each parallel microchannel includes at least one capture electrode configuration.

[0098] The microfluidic devices of the invention also typically include an integrated system. The system generally includes a computer or a computer readable medium that includes at least one instruction set for selectively activating or deactivating the at least one localized electric field in the at least one material capture portion and a controller/detector apparatus configured to receive the microfluidic device. The controller/detector apparatus typically includes a detection system and a material transport system in which the detection and transport systems are operably interfaced with the microfluidic device.

[0099] The present invention, in addition to other integrated system components, also optionally includes a microfluidic device handler for performing the methods disclosed herein. Specifically, the microfluidic device handler includes a holder configured to receive the microfluidic device, a container sampling region proximal to

the holder, and the controller. During operation of the handler, the controller directs, e.g., dipping of microfluidic device capillary or pipettor element(s) into a portion of, e.g., a microwell plate in the container sampling region. The microfluidic device handler also optionally includes a computer or a computer readable medium operably connected to the controller. The computer or the computer readable medium typically includes an instruction set for varying or selecting a rate or a mode of dipping capillary element(s) into fluid materials.

[0100] Although the devices and systems specifically illustrated herein are generally described in terms of the performance of a few or one particular operation, it will be readily appreciated from this disclosure that the flexibility of these systems permits easy integration of additional operations into these devices. For example, the devices and systems described will optionally include structures, reagents and systems for performing virtually any number of operations in addition to the operations specifically described herein. Aside from fluid handling, amplification, sequencing, and separation of sample and/or reaction components, other upstream or downstream operations include, e.g., extraction, purification, cellular activation, labeling reactions, dilution, aliquotting, labeling of components, assays and detection operations, electrokinetic or pressure-based injection of components or materials into contact with one another, or the like. Assay and detection operations include, without limitation, cell fluorescence assays, cell activity assays, receptor/ligand assays, immunoassays, or the like.

[0101] In the present invention, the materials are optionally monitored and/or detected so that, e.g., an activity can be determined. The systems described herein generally include microfluidic device handlers, as described above, in conjunction with additional instrumentation for controlling fluid transport, flow rate and direction within the devices, detection instrumentation for detecting or sensing results of the operations performed by the system, processors, e.g., computers, for instructing the controlling instrumentation in accordance with preprogrammed instructions, receiving data from the detection instrumentation, and for analyzing, storing and interpreting the data, and providing the data and interpretations in a readily accessible reporting format.

Controllers

[0102] The controllers of the integrated systems of the present invention direct dipping of capillary elements into, e.g., microwell plates to sample materials, such as enzymes and nucleic acids, fluid recirculation baths or troughs, or the like. A variety of controlling instrumentation is also optionally utilized in conjunction with the microfluidic devices and handling systems described herein, for controlling the transport, capture, concentration, direction, and motion of fluids within the devices of the present invention, e.g., by pressure-based control.

[0103] As described above, in many cases, fluid transport, capture, concentration, and direction are controlled in whole or in part, using pressure based flow systems that incorporate external or internal pressure sources to drive fluid flow. Internal sources include microfabricated pumps, e.g., diaphragm pumps, thermal pumps, and the like that have been described in the art. *See*, e.g., U.S. Patent Nos. 5,271,724, 5,277,556, and 5,375,979 and Published PCT Application Nos. WO 94/05414 and WO 97/02357. Preferably, external pressure sources are used, and applied to ports at channel termini. These applied pressures, or vacuums, generate pressure differentials across the lengths of channels to drive fluid flow through them. In the interconnected channel networks described herein, differential flow rates on volumes are optionally accomplished by applying different pressures or vacuums at multiple ports, or preferably, by applying a single vacuum at a common waste port and configuring the various channels with appropriate resistance to yield desired flow rates. Example systems are also described in USSN 09/238,467 filed 1/28/99.

[0104] Typically, the controller systems are appropriately configured to receive or interface with a microfluidic device or system element as described herein. For example, the controller and/or detector, optionally includes a stage upon which the device of the invention is mounted to facilitate appropriate interfacing between the controller and/or detector and the device. Typically, the stage includes an appropriate mounting/alignment structural element, such as a nesting well, alignment pins and/or holes, asymmetric edge structures (to facilitate proper device alignment), and the like. Many such configurations are described in the references cited herein.

[0105] The controlling instrumentation discussed above is also used to provide for electrokinetic injection or withdrawal of material downstream of the region of interest to control an upstream flow rate. The same instrumentation and techniques described above are also utilized to inject a fluid into a downstream port to function as a flow control element. A variety of electrokinetic controllers and electrical control systems which are optionally used in the present invention e.g., to effect material capture, to joule heat materials, or the like are described, e.g., in Ramsey WO 96/04547, Parce et al. WO 98/46438 and Dubrow et al., WO 98/49548, as well as a variety of other references noted herein.

Detector

[0106] The devices described herein optionally include signal detectors, e.g., which detect concentration, fluorescence, phosphorescence, radioactivity, pH, charge, absorbance, refractive index, luminescence, temperature, magnetism, mass (e.g., mass spectrometry), or the like. The detector(s) optionally monitors one or a plurality of signals from upstream and/or downstream of an assay mixing point in which, e.g., a substrate nucleic acid, an enzyme, and other reaction components are mixed. For example, the detector optionally monitors a plurality of optical signals which correspond in position to "real time" assay/separation results.

[0107] Example detectors or sensors include photomultiplier tubes, CCD arrays, optical sensors, temperature sensors, pressure sensors, pH sensors, conductivity sensors, mass sensors, scanning detectors, or the like. Materials which emit a detectable signal are optionally flowed past the detector, or, alternatively, the detector can move relative to the array to determine the position of an assay component (or, the detector can simultaneously monitor a number of spatial positions corresponding to channel regions, e.g., as in a CCD array). Each of these types of sensors is optionally readily incorporated into the microfluidic systems described herein. In these systems, such detectors are placed either within or adjacent to the microfluidic device or one or more channels, chambers or conduits of the device, such that the detector is within sensory communication with the device, channel, or chamber. The phrase "within sensory communication" of a particular region or element, as used herein, generally refers to the placement of the detector in a position such that the detector is capable of detecting the

property of the microfluidic device, a portion of the microfluidic device, or the contents of a portion of the microfluidic device, for which that detector was intended. The detector optionally includes or is operably linked to a computer, e.g., which has software for converting detector signal information into assay result information (e.g., kinetic data of modulator activity), or the like. A microfluidic system optionally employs multiple different detection systems for monitoring the output of the system. Detection systems of the present invention are used to detect and monitor the materials in a particular channel region (or other reaction detection region).

[0108] The detector optionally exists as a separate unit, but is preferably integrated with the controller system, into a single instrument. Integration of these functions into a single unit facilitates connection of these instruments with the computer (described below), by permitting the use of few or a single communication port(s) for transmitting information between the controller, the detector, and the computer.

[0109] Specific detection systems that are optionally used in the present invention include, e.g., an emission spectroscope, a fluorescence spectroscope, a phosphorescence spectroscope, a luminescence spectroscope, a spectrophotometer, a photometer, a nuclear magnetic resonance spectrometer, an electron paramagnetic resonance spectrometer, an electron spin resonance spectroscope, a turbidimeter, a nephelometer, a Raman spectroscope, a refractometer, an interferometer, an x-ray diffraction analyzer, an electron diffraction analyzer, a polarimeter, an optical rotary dispersion analyzer, a circular dichroism spectrometer, a potentiometer, a chronopotentiometer, a coulometer, an amperometer, a conductometer, a gravimeter, a mass spectrometer, a thermal gravimeter, a titrimeter, a differential scanning colorimeter, a radioactive activation analyzer, a radioactive isotopic dilution analyzer, or the like.

Computer

[0110] As noted above, the microfluidic devices and integrated systems of the present invention optionally include a computer operably connected to the controller. The computer typically includes an instruction set, e.g., for activating and/or deactivating current flow to selected field and/or capture electrodes, for regulating pressure-based fluid flow, for controlling device temperatures, for varying or selecting a rate or a mode of dipping capillary or pipettor elements into fluid materials, for sampling fluidic

materials (e.g., enzymes, substrates, reactants, materials, buffers, etc.), or the like. Additionally, either or both of the controller system and/or the detection system is/are optionally coupled to an appropriately programmed processor or computer which functions to instruct the operation of these instruments in accordance with preprogrammed or user input instructions, receive data and information from these instruments, and interpret, manipulate and report this information to the user. As such, the computer is typically appropriately coupled to one or both of these instruments (e.g., including an analog to digital or digital to analog converter as needed).

[0111] The computer typically includes appropriate software for receiving user instructions, either in the form of user input into a set parameter fields, e.g., in a GUI, or in the form of preprogrammed instructions, e.g., preprogrammed for a variety of different specific operations. The software then converts these instructions to appropriate language for instructing the operation of the fluid direction and transport controller to carry out the desired operation, e.g., varying or selecting the rate or mode of fluid and/or microfluidic device movement, controlling flow rates within microscale channels, directing xyz translation of the microfluidic device or of one or more microwell plates, or the like. The computer then receives the data from the one or more sensors/detectors included within the system, and interprets the data, either provides it in a user understood format, or uses that data to initiate further controller instructions, in accordance with the programming, e.g., such as in monitoring and control of flow rates, temperatures, applied voltages, and the like. Additionally, the software is optionally used to control, e.g., pressure or electrokinetic modulated injection or withdrawal of material.

Example Integrated System

[0112] Figure 7, Panels A, B, and C and Figure 8 provide additional details regarding example integrated systems that are optionally used to practice the methods herein. As shown, body structure **702** of microfluidic device **700** has main channel **704** disposed therein. At least microchannel **704** typically includes a capture electrode configuration as described herein. A sample or mixture of materials is optionally flowed from pipettor or capillary element **720** towards reservoir **714**, e.g., by applying a vacuum at reservoir **714** (or another point in the system) or by applying appropriate voltage gradients. Alternatively, a vacuum is applied at reservoirs **708**, **712**

or through pipettor or capillary element **720**. Additional materials, such as buffer solutions, substrate solutions, enzyme solutions, and the like, as described above are optionally flowed from wells **708** or **712** and into main channel **704**. Flow from these wells is optionally performed by modulating fluid pressure, or by electrokinetic approaches as described (or both). Pressure-based flow is preferred. As fluid is added to main channel **704**, e.g., from reservoir **708**, the flow rate increases. The flow rate is optionally reduced by flowing a portion of the fluid from main channel **704** into flow reduction channel **706** or **710**. Fluid flow is typically dispersion-controlled as described herein. The arrangement of channels depicted in Figure 7 is only one possible arrangement out of many which are appropriate and available for use in the present invention. Alternatives are provided in Figures 1, 2, 3, 4, 5, and 6. Additional alternatives can be devised, e.g., by combining the microfluidic elements described herein, e.g., flow reduction channels, with other microfluidic devices described in the patents and applications referenced herein. Furthermore the elements of Figures 1, 2, 3, 4, 5, and/or 6 are optionally recombined to provide alternative configurations.

[0113] Samples and materials are optionally flowed from the enumerated wells or from a source external to the body structure. As depicted, the integrated system optionally includes pipettor or capillary element **820**, e.g., protruding from body **802**, for accessing a source of materials external to the microfluidic system. Typically, the external source is a microtiter dish or other convenient storage medium. For example, as depicted in Figure 8, pipettor or capillary element **820** can access microwell plate **808**, which includes sample materials, buffers, substrate solutions, enzyme solutions, and the like, in the wells of the plate.

[0114] Detector **806** is in sensory communication with main channel **704**, detecting signals resulting, e.g., from labeled materials flowing through the detection region. Detector **806** is optionally coupled to any of the channels or regions of the device where detection is desired. Detector **806** is operably linked to computer **804**, which digitizes, stores, and manipulates signal information detected by detector **806**, e.g., using any of the instructions described above, e.g., or any other instruction set, e.g., for determining concentration, molecular weight or identity, or the like.

[0115] Fluid direction system **802** controls voltage, pressure, or both, e.g., at the wells of the systems or through the channels of the system, or at vacuum couplings fluidly coupled to channel **704** or other channel described above. Optionally, as depicted, computer **804** controls fluid direction system **802**. In one set of embodiments, computer **804** uses signal information to select further parameters for the microfluidic system. For example, upon detecting the presence of a component of interest in a sample from microwell plate **808**, the computer optionally directs addition of a potential modulator of component of interest into the system.

[0116] Electrical control system **810** controls the delivery of AC and/or DC to the field electrodes, capture electrodes, capture electrode pairs of the capture electrode configurations described herein, e.g., to control the dispersion of materials during fluid transport, to regulate temperatures, or the like within the microchannels or other cavities of microfluidic device **700**. Electrical control system **810** is also typically operably connected to computer **804**, which directs the activation and/or deactivation of current flow from electrical control system **810**.

[0117] Although not shown, a microfluidic device handler is also typically included in the integrated systems of the present invention. Microfluidic device handlers generally control, e.g., the xyz translation of microfluidic device **700** relative to microwell plate **808**, or other system components, under the direction of computer **804** to which device handlers are typically operably connected.

KITS

[0118] Generally, the microfluidic devices described herein are optionally packaged to include reagents for performing the device's preferred function. For example, the kits can include any of microfluidic devices described along with assay components, reagents, sample materials, particle sets, salts, separation matrices, control/calibrating materials, or the like. Such kits also typically include appropriate instructions for using the devices and reagents, and in cases where reagents are not predisposed in the devices themselves, with appropriate instructions for introducing the reagents into the channels and/or chambers of the device. In this latter case, these kits optionally include special ancillary devices for introducing materials into the microfluidic systems, e.g., appropriately configured syringes/pumps, or the like (in one preferred

embodiment, the device itself comprises a pipettor element, such as an electropipettor for introducing material into channels and chambers within the device). In the former case, such kits typically include a microfluidic device with necessary reagents predisposed in the channels/chambers of the device. Generally, such reagents are provided in a stabilized form, so as to prevent degradation or other loss during prolonged storage, e.g., from leakage. A number of stabilizing processes are widely used for reagents that are to be stored, such as the inclusion of chemical stabilizers (i.e., enzymatic inhibitors, microcides/bacteriostats, anticoagulants), the physical stabilization of the material, e.g., through immobilization on a solid support, entrapment in a matrix (i.e., a gel), lyophilization, or the like. Kits also optionally include packaging materials or containers for holding a microfluidic device, system or reagent elements.

[0119] While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be clear to one skilled in the art from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention. For example, all the techniques and apparatus described above may be used in various combinations. All publications, patents, patent applications, or other documents cited in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication, patent, patent application, or other document were individually indicated to be incorporated by reference for all purposes.